Operation and Design Principles of Biological Molecular Machines

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Keywords Single-Molecule Biophysics, Molecular Machines, Molecular Motors

Activity of life is supported by various molecular machines made of proteins and nucleic acids. These biological molecular machines show high performance such as reaction specificity and energy conversion efficiency, and are superior to man-made machines in some aspects.

One of the representatives of the molecular machines is linear and rotary molecular motors (Figure 1). Molecular motors generate mechanical forces and torques that drive their unidirectional motions from the energy of chemical reaction or the potential energy.

We will unveil operation principles of biological molecular motors and machines with single-molecule techniques based on optical microscopy. We will also try to create new biological molecular motors and machines to understand their design principles. Our ultimate goal is controlling living organisms with created molecular machines.

Figure 1. A linear molecular motor chitinase. Chitinase moves on the substrate crystalline chitin unidirectionally and processively, driven by the energy of hydrolysis of the chain end of the chitin.

Selected Publications

- R. Iino and H. Noji, "Intersubunit Coordination and Cooperativity in Ring-Shaped NTPases," *Curr. Opin. Struct. Biol.* **23**, 229–234 (2013).
- R. Iino and H. Noji, "Operation Mechanism of F_oF_1-Adenosine Triphosphate Synthase Revealed by Its Structure and Dynamics," *IUBMB Life* **65**, 238–246 (2013).
- Y. Shibafuji, A. Nakamura, T. Uchihashi, N. Sugimoto, S. Fukuda, H. Watanabe, M. Samejima, T. Ando, H. Noji, A. Koivula, K. Igarashi and R. Iino, "Single-Molecule Imaging Analysis of Elementary Reaction Steps of *Trichoderma reesei* Cellobiohydrolase I (Cel7A) Hydrolyzing Crystalline Cellulose I_α and III_I," *J. Biol. Chem.* **289**, 14056–14065 (2014).
- Y. Minagawa, H. Ueno, M. Hara, Y. Ishizuka-Katsura, N. Ohsawa, T. Terada, M. Shirouzu, S. Yokoyama, I. Yamato, E. Muneyuki, H. Noji, T. Murata and R. Iino, "Basic Properties of Rotary Dynamics of the Molecular Motor *Enterococcus hirae* V_1-ATPase," *J. Biol. Chem.* **288**, 32700–32707 (2013).
- R. Watanabe, K. V. Tabata, R. Iino, H. Ueno, M. Iwamoto, S. Oiki and H. Noji, "Biased Brownian Stepping Rotation of F_oF_1-ATP Synthase Driven by Proton Motive Force," *Nat. Commun.* **4**, 1631 (2013).
- T. Uchihashi, R. Iino, T. Ando and H. Noji, "High-Speed Atomic Force Microscopy Reveals Rotary Catalysis of Rotorless F_1-ATPase," *Science* **333**, 755–758 (2011).

1. Rotary Dynamics of *Enterococcus hirae* V_1-ATPase[1)]

V-ATPases are rotary molecular motors that generally function as proton pumps. We characterized the rotary dynamics of the V_1 moiety of *Enterococcus hirae* V-ATPase (EhV_1, Figure 2A) using single-molecule analysis employing a load-free probe (Figure 2B). EhV_1 rotated in a counterclockwise direction, exhibiting two distinct rotational states, namely clear and unclear, suggesting unstable interactions between the rotor and stator. The clear state was analyzed in detail to obtain kinetic parameters. The rotation rates obeyed Michaelis-Menten kinetics with a maximal rotation rate (V_{max}) of 107 revolutions/s and a Michaelis constant (K_m) of 154 μM at 26 °C. At all ATP concentrations tested, EhV_1 showed only three pauses separated by 120°/turn, and no substeps were resolved, as was the case with *Thermus thermophilus* V_1-ATPase (TtV_1). At 10 μM ATP ($<<K_m$), the distribution of the durations of the ATP-waiting pause fit well with a single-exponential decay function. The second-order binding rate constant for ATP was 2.3×10^6 M^{-1} s^{-1}. At 40 mM ATP ($>>K_m$), the distribution of the durations of the catalytic pause was reproduced by a consecutive reaction with two time constants of 2.6 and 0.5 ms. These kinetic parameters were similar to those of TtV_1. Our results identified the common properties of rotary catalysis of V_1-ATPases that are distinct from those of F_1-ATPases and furthered our understanding of the general mechanisms of rotary molecular motors.

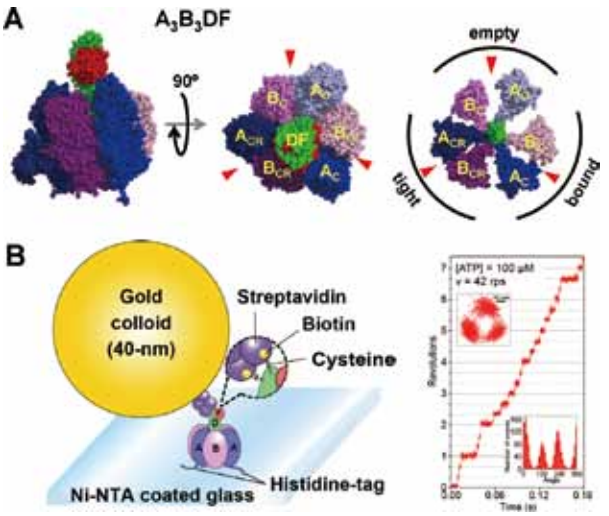

Figure 2. (A) Crystal structure of EhV_1. (B) Left, Schematics of the single-molecule rotation assay of EhV_1. Right, Example of the rotary motion.

2. Mechanism of Different Susceptibilities of Cellulose I_α and III_I to Hydrolysis by a Linear Molecular Motor Cellulase[2)]

A cellulase, *Trichoderma reesei* Cel7A (*Tr*Cel7A) is a linear molecular motor that directly hydrolyzes crystalline celluloses into water-soluble cellobioses. It has recently drawn attention as a tool that could be used to convert cellulosic materials into biofuel. However, detailed mechanisms of action, including elementary reaction steps such as binding, processive hydrolysis, and dissociation, have not been thoroughly explored because of the inherent challenges associated with monitoring reactions occurring at the solid/liquid interface. The crystalline cellulose I_α and III_I were previously reported as substrates with different crystalline forms and different susceptibilities to hydrolysis by *Tr*Cel7A. We observed that different susceptibilities of cellulose I_α and III_I are highly dependent on enzyme concentration, and at nanomolar enzyme concentration, *Tr*Cel7A shows similar rates of hydrolysis against cellulose I_α and III_I. Using single-molecule fluorescence microscopy and high-speed atomic force microscopy, we also determined kinetic constants of the elementary reaction steps for *Tr*Cel7A against cellulose I_α and III_I. These measurements were performed at picomolar enzyme concentration in which density of *Tr*Cel7A on crystalline cellulose was very low. Under this condition, *Tr*Cel7A displayed similar binding and dissociation rate constants for cellulose I_α and III_I and similar fractions of productive binding on cellulose I_α and III_I. Furthermore, once productively bound, *Tr*Cel7A processively hydrolyzes and moves along cellulose I_α and III_I with similar translational rates. With structural models of cellulose I_α and III_I, we proposed that different susceptibilities at high *Tr*Cel7A concentration arise from surface properties of substrate, including ratio of hydrophobic surface and number of available lanes (Figure 3).

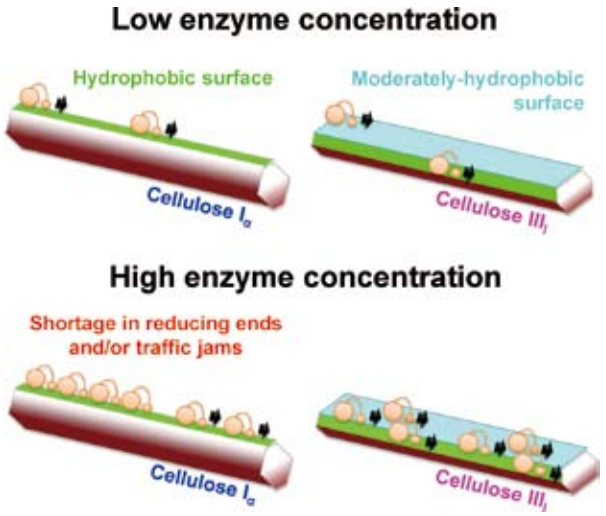

Figure 3. A model of different susceptibilities of cellulose I_α and III_I to hydrolysis by *Tr*Cel7A.

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2) Y. Shibafuji, A. Nakamura, T. Uchihashi, N. Sugimoto, S. Fukuda, H. Watanabe, M. Samejima, T. Ando, H. Noji, A. Koivula, K. Igarashi and R. Iino, *J. Biol. Chem.* **289**, 14056–14065 (2014).